

## Design and Synthesis of 1-Heteroaryl-3-(1-benzyl-4-piperidinyl)propan-1-one Derivatives as Potent, Selective Acetylcholinesterase Inhibitors

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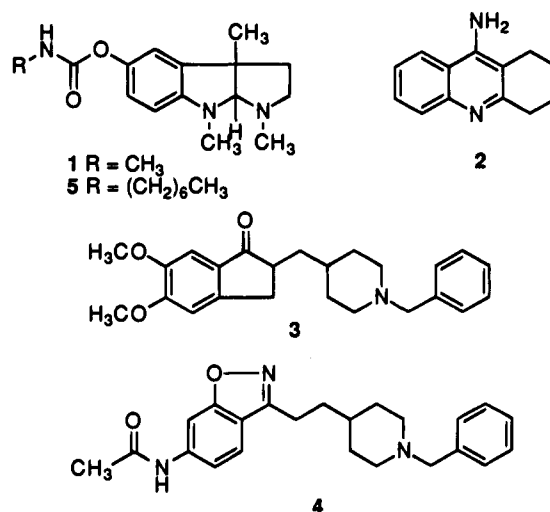
Herein is described the synthesis and structure-activity relationship of a novel series of aromatic and heteroaromatic 3-(1-benzyl-4-piperidinyl)propan-1-one derivatives that display potent and selective inhibition of the enzyme acetylcholinesterase (AChE). 1-(2-Methyl-6-benzothiazolyl)-3-(*N*-benzyl-4-piperidinyl)propan-1-one hydrochloride, **6d**, is one of the most active compounds within this series exhibiting an IC<sub>50</sub> for the inhibition of the AChE enzyme equal to 6.8 nM. Compound **6d** has shown a dose-dependent elevation of total acetylcholine (ACh) levels in the mouse forebrain with an oral ED<sub>50</sub> = 9.8 mg/kg. In addition, *in vivo* microdialysis experiments in the rat demonstrate that **6d** increases extracellular ACh (100% over basal) 1-3 h postdose with an oral ED<sub>50</sub> = 4.8 mg/kg.

A fundamental biological deficit associated with senile dementia of the Alzheimer's type (SDAT) is the substantially reduced activity throughout the cerebral cortex of the enzyme choline acetyltransferase (ChAT).<sup>1</sup> This enzyme is responsible for the synthesis of the neurotransmitter acetylcholine (ACh). Based on the above observation, the cholinergic hypothesis has evolved, which postulates that increasing ACh levels in SDAT patients will enhance cognitive function.<sup>2</sup> Acetylcholinesterase (AChE) is an enzyme responsible for the breakdown of ACh within the synaptic cleft. It has been demonstrated that cognitive function can be improved in SDAT patients by inhibiting AChE.<sup>3,4</sup> Therefore, there has been a major effort to identify selective AChE inhibitors for the treatment of SDAT. There are three major structural classes of AChE inhibitors which have undergone extensive human profiling (Chart 1): the dihydroindole series represented by physostigmine (**1**),<sup>3</sup> the tetrahydroacridine series represented by tacrine (**2**),<sup>4</sup> and the benzylpiperidines such as **3**<sup>5</sup> and **4**.<sup>6</sup>

Physostigmine (**1**) is a potent AChE inhibitor<sup>7</sup> (Table 1) but suffers from a poor pharmacokinetic profile.<sup>8</sup> Therefore, its use in SDAT patients has been rather limited, and considerable effort has been placed in the formulation of **1** to improve the pharmacokinetic profile.<sup>9</sup> Heptylphysostigmine (**5**)<sup>10</sup> has been claimed to have an improved pharmacokinetic profile as compared to **1** and to be less toxic.

Tacrine, **2**, a less potent AChE inhibitor<sup>7</sup> than either physostigmine or the benzylpiperidine derivatives **3**<sup>5</sup> and **4**<sup>6</sup> (Table 1), has been approved for treating Alzheimer's patients. Compound **2** potently inhibits a second enzyme, butyrylcholinesterase (BuChE).<sup>11</sup> BuChE is found in both plasma and brain, and it has been postulated that inhibition of BuChE may lead to adverse peripheral side effects.<sup>11</sup> In addition, compound **2** has a complicated pharmacokinetic profile (at least five major metabolites)<sup>12</sup> and is known to cause hepatotoxic side effects in a significant number of patients.<sup>4b,c</sup> The nonselectivity of **2** and its potential to induce hepato-

Chart 1



toxic side effects suggest that selective, nonhepatotoxic AChE inhibitors would provide a significant improvement in AChE inhibition therapy.

The benzylpiperidine series represented by structures **3**<sup>5</sup> and **4**<sup>6</sup> have been shown to be potent and selective AChE inhibitors. In addition, compound **3** ( $t_{1/2} = 40$  h)<sup>13</sup> presents a significantly different pharmacokinetic profile in man than either **1** or **2**. The interesting profile of the benzylpiperidines prompted an investigation initially to define the minimum requirements for AChE inhibition within this series. Removal of all aromatic substituents from **3** and **4**, as well as the enantiomeric center in compound **3**,<sup>14</sup> afforded the initial target structure **6a** (Scheme 1). Herein we report a novel series of heteroaryl benzylpiperidines that are potent and selective AChE inhibitors.

### Chemistry

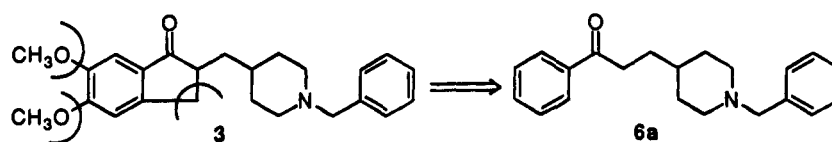
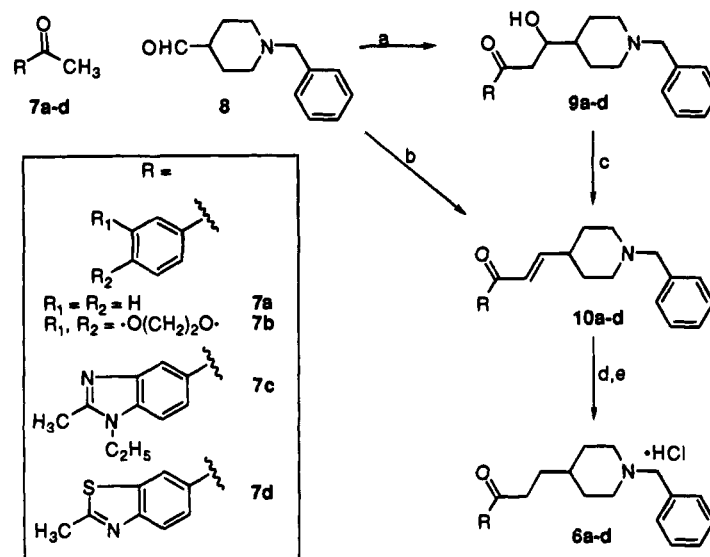
Two general methods were used to synthesize compounds **6a-d**, **19**, and **20** depicted in Table 1. The first method, outlined in Scheme 2, involved the condensation of heteroaromatic methyl ketones **7a-d** with

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## Scheme 1

Scheme 2<sup>a</sup>

<sup>a</sup> Reagents: (a) lithium bis(trimethylsilyl)amide, THF,  $-78^{\circ}\text{C}$ ,  $\text{H}_2\text{O}$ ; (b) lithium bis(trimethylsilyl)amide, THF,  $-78^{\circ}\text{C}$  to rt, 1 N HCl; (c) ethyl(carboxysulfamoyl)triethylammonium hydroxide inner salt, THF,  $50^{\circ}\text{C}$ , or *p*-TsOH, benzene, reflux ( $-\text{H}_2\text{O}$ ); (d)  $\text{H}_2$ ,  $\text{PtO}_2$ , EtOH; (e) HCl/EtOAc.

**Table 1.** Comparison of the *in Vitro* Enzyme Inhibition of AChE<sup>a</sup> and BuChE<sup>b</sup> with Compounds 6a–d, 19, and 20 and Known AChE Inhibitors

compound	IC <sub>50</sub> (nM) <sup>c</sup>	
	AChE	BuChE
6a	305 ± 45	4300 ± 2300
6b	30 ± 2.8	4800 ± 2100
6c	4.3 ± 0.6	4200 ± 1600
6d	6.8 ± 1.4	3400 ± 1400
19	12.0 ± 1.0	6200 ± 500
20	33.0 ± 4.7	890 ± 80
1 (physostigmine)	18.9 ± 5.0	73 ± 6.0
2 (tacrine)	267 ± 135	6.0 ± 1.0
3	8.0 ± 3.4	2900 ± 60

<sup>a</sup> Source of AChE: human erythrocytes. <sup>b</sup> Source of BuChE: human serum. <sup>c</sup> IC<sub>50</sub> values are the mean ± standard deviation of at least three assays.

1-benzyl-4-formylpiperidine (**8**),<sup>15</sup> using basic conditions (lithium bis(trimethylsilyl)amide, THF,  $-78^{\circ}\text{C}$ ). After approximately 45 min the reaction was quenched with water at  $-78^{\circ}\text{C}$  and the mixture warmed to room temperature to afford alcohols **9a–d** in good to excellent overall yield. Dehydration of alcohols **9a–d** was readily accomplished using ethyl(carboxysulfamoyl)triethylammonium hydroxide inner salt<sup>16</sup> (generated *in situ*) or refluxing alcohols **9a–d** in benzene with a catalytic amount of TsOH (Dean–Stark trap) to afford the  $\alpha,\beta$ -unsaturated ketone derivatives **10a–d** as a mixture of *cis* and *trans* double-bond isomers. The  $\alpha,\beta$ -unsaturated ketone derivatives **10a–d** could also be obtained directly from the condensation of ketones **7a–d** with aldehyde **8** by warming the condensation reaction to room temperature prior to quenching. However, yields of ketones **10a–d** varied significantly; considerable amounts of starting ketones **7a–d** were noted in the reaction mixtures. Presumably alcohols **9a–d** undergo a reverse

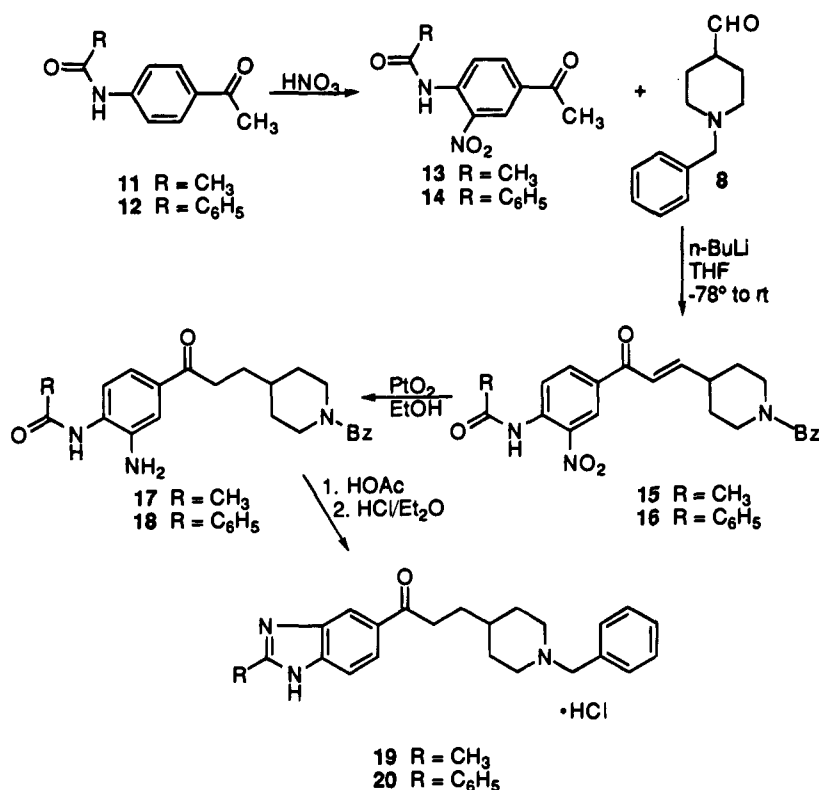
aldol reaction as the reaction mixture is warmed to room temperature. Hydrogenation of compounds **10a–d** ( $\text{PtO}_2/\text{EtOH}$ ) affords the desired compounds **6a–d**. Compounds **6a–d** were converted to their respective hydrochloride salts for biological analysis.

Compounds **19** and **20** (Table 1) were prepared as outlined in Scheme 3. This straightforward route generates the 1*H*-benzimidazole in the last synthetic step and avoids all reactions with the 1*H*-benzimidazole under basic conditions. Nitration of the appropriately *N*-substituted *p*-aminoacetophenone **11** or **12** affords a 50% yield of crystalline nitro compounds **13** and **14**. Condensation of ketones **13** and **14** with aldehyde **8** (to afford **15** and **16**) followed by simultaneous reduction of the  $\alpha,\beta$ -unsaturated ketone double bond and aromatic nitro group yields the aminoacetophenone derivatives **17** and **18**. The benzimidazole is then formed by refluxing **17** and **18** in acetic acid to produce compounds **19** and **20**. The hydrochloride salts of **19** and **20** were prepared for biological screening.

## Biological Results

Table 1 lists the *in vitro* inhibition of AChE and BuChE by compounds **6a–d**, **19**, and **20**, as well as that for compounds **1–3**. As shown, compound **6a** possessed significantly reduced potency as compared to **3** in inhibiting AChE. However, introduction of a dimethylenedioxy substituent onto the phenyl ring (compound **6b**; Table 1) increased AChE inhibition activity approximately 10-fold. Furthermore, AChE inhibition potency could be improved by replacing the phenyl ring with substituted benzimidazoles (compounds **6c**, **19**, and **20**) or thiazole (compound **6d**) substituents. As shown in Table 1, compounds **6a–d**, **19**, and **20** demonstrate

## Scheme 3



between 27- (20) and 975- (6c) fold selectivity for inhibition of AChE as compared to BuChE. Compound 6d was selected from this group for further *in vivo* profiling.

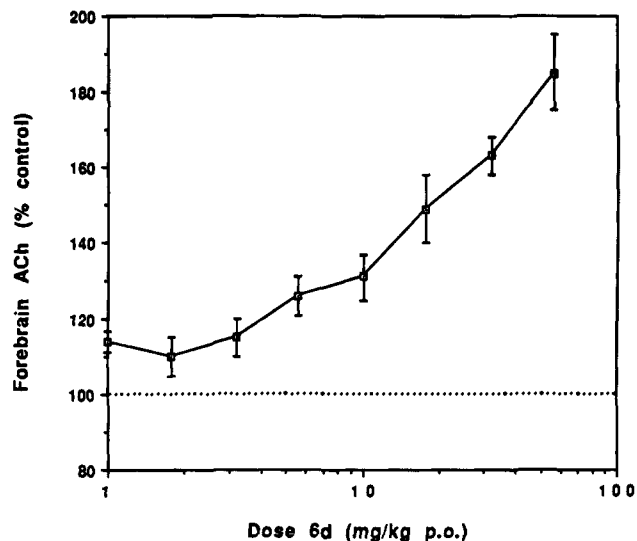
To test the *in vivo* effects of compound 6d, ACh metabolism in rodents was examined. In mice, 6d produced a robust elevation in forebrain ACh concentration at 1 h postdose. This effect was dose-related, with an ED<sub>50</sub> of 9.8 mg/kg po (Figure 1). A full range of central and peripheral cholinergic effects (tremors, hypothermia, sedation, salivation, lacrimation) was observed in mice at doses greater than 32 mg/kg po (data not shown).

*In vivo* microdialysis was used to measure the effects of compound 6d on the extracellular accumulation of ACh in the striatum of conscious rats. As shown in Figure 2, oral administration of compound 6d produced a dose-related increase in extracellular ACh in the striatum for a period of 1–3 h postdose. The dose required to produce a doubling of ACh levels during the 3 h following drug administration (100% increase over basal) was calculated to be 4.8 mg/kg. However, higher doses of 6d led to very large increases in accumulation of extracellular ACh (Figure 2).

The ability of compound 6d to inhibit AChE potently and selectively, coupled with its ability to raise mouse brain ACh levels following oral administration, suggests that this structurally distinct compound may possess advantages in the treatment of SDAT as compared to presently known AChE inhibitors.

### Experimental Section

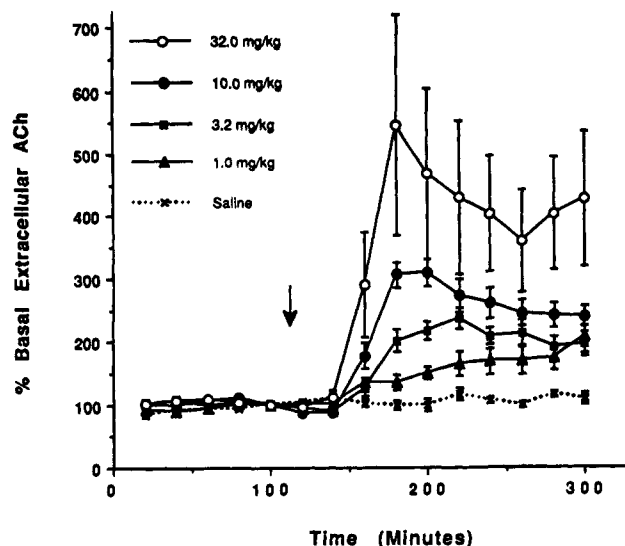
Melting points were obtained on a Electrothermal digital melting point apparatus and are uncorrected. NMR spectra were obtained on a Varian XL-300 or a Bruker AM-300 spectrometer, with tetramethylsilane as an internal standard. FAB ionization mass spectra (M<sup>+</sup> data reported) were obtained



**Figure 1.** ACh levels in mouse forebrain following oral administration of 6d. This effect was dose-related over the range 1–56 mg/kg po; at doses greater than 56 mg/kg po, excessive mortality (>50%) was observed. ACh levels in the control group (vehicle-injected) were 28.6 ± 0.9 nmol/g of tissue. The ED<sub>50</sub> for ACh elevation, defined as a 40% elevation in forebrain ACh content, was 9.8 mg/kg (95% confidence intervals: 8.1, 11.8). Data are means ± SEM from a single representative experiment where *n* = 5–8 animals/dose.

on a Kratos Concept mass spectrometer, and EI high- or low-resolution mass spectra were obtained on a Kratos Profile instrument. TLC analysis was carried out on EM Kieselgel 60 F<sub>254</sub> 5 × 20 cm plates. Column chromatography used EM Science silica gel 60 (230–400 mesh) as the solid phase. Elemental analyses were obtained from Schwarzkopf Microanalytical Laboratory, Woodside, NY.

**General Preparation of Alcohols 9a–d. Preparation of 3-(1-Benzylpiperidin-4-yl)-1-(1-ethyl-2-methyl-1*H*-benzimidazol-5-yl)-3-hydroxypropan-1-one, 9c.** A solution of 2.5 mL (2.5 mmol) of lithium bis(trimethylsilyl)amide (1 M



**Figure 2.** *In vivo* microdialysis in rat striatum following oral administration of **6d**. Base line samples were collected for 2 h following insertion of probes, whereupon drug was administered (arrow) and sample collection continued for 3 h. The dose required to produce a doubling of ACh levels during the 3 h following drug administration (100% increase over basal) was calculated to be 4.8 mg/kg (95% confidence interval: 3.1, 7.5). Data represent means  $\pm$  SEM for 6–10 animals/dose.

solution in THF) in 10 mL of THF was cooled to  $-78^{\circ}\text{C}$  in a flame-dried round bottom flask under a nitrogen atmosphere. To this solution was added dropwise a solution of 500 mg (2.47 mmol) of 1-ethyl-2-methyl-5-benzimidazolyl methyl ketone (**7c**)<sup>17</sup> dissolved in 5 mL of THF. The mixture was stirred at  $-78^{\circ}\text{C}$  for 15 min. To this mixture was added dropwise a solution of 501 mg (2.47 mmol) of 1-benzyl-4-formylpiperidine (**8**) in 5 mL of THF. After the addition was complete, the reaction mixture was stirred at  $-78^{\circ}\text{C}$  for 45 min. The reaction was then quenched with 25 mL of  $\text{H}_2\text{O}$  and the mixture warmed to room temperature. The pH of the reaction mixture was adjusted to 4.0 and the mixture extracted with EtOAc. The water layer was then adjusted to pH = 9.5 and extracted with EtOAc. The pH = 9.5 EtOAc extracts were combined, dried, and evaporated to yield 800 mg of solid residue. This residue was chromatographed on 30 g of silica gel using 10:1  $\text{CHCl}_3:\text{CH}_3\text{OH}$  as the eluant to yield 650 mg (65%) of **9c** as a white crystalline solid: mp =  $148\text{--}149^{\circ}\text{C}$ ; TLC (10:1  $\text{CHCl}_3:\text{CH}_3\text{OH}$ )  $R_f$  = 0.50; MS  $m/e$  = 406 ( $\text{M}^+$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.28 (s, 1H), 7.91 (d,  $J$  = 2 Hz, 1H), 7.2–7.4 (m, 6H), 4.20 (q,  $J$  = 2 Hz, 2H), 4.0 (m, 1H), 3.55 (s, 2H), 3.30 (d,  $J$  = 7 Hz, 1H), 2.90–3.16 (m, 3H), 2.65 (s, 3H), 1.70–2.10 (m, 4H), 1.67 (m, 1H), 1.48 (m, 2H), 1.42 (t,  $J$  = 3 Hz, 3H).

**3-(1-Benzylpiperidin-4-yl)-3-hydroxy-1-phenylpropan-1-one, 9a:** similarly prepared, clear oil (65% yield from acetophenone); MS  $m/e$  = 324 ( $\text{M}^+$ ); TLC (1:1 EtOAc:hexanes)  $R_f$  = 0.1;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.91 (d,  $J$  = 2 Hz, 2H), 7.52 (m, 1H), 7.42 (t,  $J$  = 2 Hz, 3H), 7.2–7.36 (m, 4H), 3.98 (m, 1H), 3.48 (s, 2H), 3.16 (d,  $J$  = 6 Hz, 1H), 2.86–3.08 (m, 3H), 1.8–2.0 (m, 3H), 1.64 (m, 1H), 1.3–1.5 (m, 3H).

**3-(1-Benzylpiperidin-4-yl)-1-(2,3-dihydrobenzo[1,4]-dioxin-6-yl)-3-hydroxypropan-1-one, 9b:** 64.8% yield from 1,4-benzodioxin-6-yl methyl ketone; mp =  $160\text{--}162^{\circ}\text{C}$ ; MS  $m/e$  = 382 ( $\text{M}^+$ ); TLC (10:1  $\text{CHCl}_3:\text{CH}_3\text{OH}$ )  $R_f$  = 0.55;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.46 (m, 2H), 7.18–7.38 (m, 5H), 6.87 (d,  $J$  = 3 Hz, 1H), 4.30 (m, 4H), 3.92 (m, 1H), 3.49 (s, 2H), 3.10 (d,  $J$  = 7 Hz, 1H), 2.80–3.0 (m, 3H), 1.8–2.0 (m, 3H), 1.60 (m, 1H), 1.30–1.50 (m, 3H).

**3-(1-Benzylpiperidin-4-yl)-3-hydroxy-1-(2-methylbenzothiazol-6-yl)propan-1-one, 9d:** crystalline solid (90% yield from 2-methylbenzothiazol-5-yl methyl ketone<sup>14</sup>); mp =  $157\text{--}158^{\circ}\text{C}$ ; MS  $m/e$  = 395 ( $\text{M}^+$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  200.1, 171.5, 156.6, 138.6, 136.1, 133.4, 129.2 (2), 128.2 (2), 126.9, 125.9, 122.4, 122.2, 71.39, 63.4, 53.7, 53.6, 42.3, 41.4, 28.2, 28.14, 20.5;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.45 (s, 1H), 8.0 (dd, 2H), 7.2–

7.38 (m, 5H), 4.02 (m, 1H), 3.48 (s, 2H), 3.05–3.3 (m, 4H), 2.93 (m, 1H), 2.85 (s, 3H), 1.8–2.0 (m, 3H), 1.68 (m, 1H), 1.35–1.68 (m, 3H).

**Dehydration of Alcohols 9a–c Using Ethyl(carboxysulfamoyl)triethylammonium Hydroxide Inner Salt. Preparation of 1-(1-Ethyl-2-methyl-5-benzimidazolyl)-3-(*N*-benzyl-4-piperidinyl)-2-propen-1-one, 10c.** *In situ* generation of ethyl(carboxysulfamoyl)triethylammonium hydroxide inner salt was accomplished as follows: A solution of 0.086 mL (1.0 mmol) of chlorosulfonyl isocyanate was dissolved in 5 mL of anhydrous THF under a nitrogen atmosphere, and cooled to  $5^{\circ}\text{C}$ . To this was added 0.040 mL (1.0 mmol) of  $\text{CH}_3\text{OH}$ , and the mixture was stirred at  $5^{\circ}\text{C}$  for 15 min. To this mixture was added 0.276 mL (2.0 mmol) of triethylamine, and the resulting cloudy suspension was stirred for 30 min at  $5^{\circ}\text{C}$ . A solution of 194 mg (0.479 mmol) of alcohol **9c** in 5 mL of THF was then added to the *in situ*-generated inner salt, the ice bath removed, and the reaction mixture refluxed for 0.5 h. The reaction mixture was cooled to room temperature, the reaction quenched with  $\text{H}_2\text{O}$ , and the mixture extracted with EtOAc. The EtOAc layer was washed with saturated  $\text{NaHCO}_3$ , dried, and evaporated. The residue was chromatographed on 15 g of silica gel using 10:1  $\text{CHCl}_3:\text{CH}_3\text{OH}$  as the eluant. Appropriate fractions were combined to produce 165 mg (89% yield) of alcohol **10c** as an oil: MS  $m/e$  = 388 ( $\text{M}^+$ ); TLC (10:1  $\text{CHCl}_3:\text{CH}_3\text{OH}$ )  $R_f$  = 0.58;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.22 (s, 1H), 7.88 (d, 1H), 7.30 (m, 6H), 6.90 (m, 2H), 4.14 (q, 2H), 3.50 (s, 2H), 3.05 (m, 2H), 2.85 (m, 2H), 2.61 (s, 3H), 2.12 (m, 1H), 2.04 (m, 2H), 1.74 (m, 2H), 1.38 (m, 2H), 1.38 (t, 3H).

**3-(1-Benzylpiperidin-4-yl)-1-phenylprop-2-en-1-one, 10a:** similarly prepared, oil (88% yield); MS  $m/e$  = 306 ( $\text{M}^+$ ); TLC (1:1 EtOAc:hexanes)  $R_f$  = 0.60;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.9 (d,  $J$  = 3 Hz, 2H), 7.36–7.56 (m, 3H), 7.20–7.36 (m, 5H), 6.88–7.02 (m, 2H), 3.64 (s, 2H), 3.02 (brd, 2H), 2.28 (m, 1H), 2.16 (brt, 2H), 1.5–1.9 (m, 4H).

**3-(1-Benzylpiperidin-4-yl)-1-(2,3-dihydrobenzo[1,4]-dioxin-6-yl)prop-2-en-1-one, 10b:** oil (78% yield); MS  $m/e$  = 364 ( $\text{M}^+$ ); TLC (2:1 EtOAc:hexanes)  $R_f$  = 0.50;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.48 (s, 1H), 7.46 (d,  $J$  = 3 Hz, 1H), 7.20–7.38 (m, 6H), 6.72–7.0 (m, 2H), 4.28 (dd, 4H), 3.54 (s, 2H), 2.94 (brd,  $J$  = 5 Hz, 2H), 2.20 (m, 1H), 2.03 (t,  $J$  = 5 Hz, 2H), 1.76 (m, 2H), 1.58 (m, 2H).

**Dehydration of 9d Using *p*-Toluenesulfonic Acid, Benzene, and Reflux. Preparation of 1-(2-Methyl-6-benzothiazolyl)-3-(*N*-benzyl-4-piperidinyl)-2-propen-1-one, 10d.** A mixture of 755 mg (1.92 mmol) of **9d** in 20 mL of benzene containing 40 mg (0.21 mmol) of *p*-toluenesulfonic acid was refluxed for 12 h, in the presence of a Dean–Stark apparatus. The reaction mixture was cooled to room temperature and diluted with EtOAc and  $\text{H}_2\text{O}$ . The pH of this mixture was adjusted to 9.5 with 1 N NaOH. The organic layer was separated from the  $\text{H}_2\text{O}$  layer, extracted with saturated  $\text{NaHCO}_3$  and saturated NaCl, dried, and evaporated. The residue was chromatographed on silica gel using EtOAc as the eluant. Appropriate fractions were combined and evaporated to yield **10d** (67% yield) as a white solid: mp =  $146\text{--}149^{\circ}\text{C}$ . MS calcd for  $\text{C}_{23}\text{H}_{24}\text{N}_2\text{OS}$   $m/e$  =  $376.1600 \pm 2.6$  ppm; TLC (10:1  $\text{CH}_2\text{Cl}_2:\text{CH}_3\text{OH}$ )  $R_f$  = 0.63;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.42 (s, 1H), 7.9 (m, 2H), 7.35–7.5 (m, 5H), 6.8–7.1 (m, 2H), 3.52 (s, 2H), 2.95 (m, 2H), 2.8 (s, 3H), 1.4–2.5 (m, 7H).

**Direct Preparation of 1-(1-Ethyl-2-methyl-5-benzimidazolyl)-3-(*N*-benzyl-4-piperidinyl)-2-propen-1-one, 10c.** A mixture of 100 mg (0.5 mmol) of 1-ethyl-2-methylbenzimidazol-5-yl methyl ketone (**7c**) and 100 mg (0.5 mmol) of 4-formyl-*N*-benzylpiperidine (**8**) in 10 mL of THF was cooled to  $-78^{\circ}\text{C}$  under a nitrogen atmosphere. To this mixture was added dropwise 0.5 mL (0.5 mmol) of a 1 M solution of lithium bis(trimethylsilyl)amide in THF. The reaction mixture was stirred at  $-78^{\circ}\text{C}$  for 1 h and then warmed to room temperature. The reaction was quenched with 10 mL of water, and the pH was adjusted to 2.0 with 1 N HCl. The mixture was extracted with 15 mL of EtOAc. The pH of the water layer was then sequentially adjusted to 3.0, 4.0, 5.0, 6.5, and 8.5 with 1 N NaOH, each time extracting with 15 mL of EtOAc.

The EtOAc extracts at pH = 5.0 and 6.5 were combined, dried with Na<sub>2</sub>SO<sub>4</sub>, and evaporated to yield 50 mg (26%) of ketone derivative **10c** as an oil. This compound was identical to **10c** prepared above. Similarly prepared was 1-(2-methyl-6-benzothiazolyl)-3-(*N*-benzyl-4-piperidinyl)-2-propen-1-one, **10d** (28% yield).

**Preparation of 1-(1-Ethyl-2-methyl-5-benzimidazolyl)-3-(*N*-benzyl-4-piperidinyl)propan-1-one Hydrochloride, **6c**.** To a solution of 0.14 g (0.36 mmol) of ketone **10c** in 20 mL of ethanol was added 10 mg of PtO<sub>2</sub>, and the mixture was hydrogenated at 50 psi for 1 h. The reaction mixture was filtered and the ethanol solvent evaporated. The residue was suspended in 50 mL of a 1:1 mixture of EtOAc:H<sub>2</sub>O and the pH adjusted to 8.5 with 1 N NaOH. The EtOAc layer was dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated to yield 100 mg (72%) of ketone **6c** as an oil: TLC (10:1 CHCl<sub>3</sub>:CH<sub>3</sub>OH) *R<sub>f</sub>* = 0.64; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 8.26 (s, 1H), 7.92 (d, *J* = 3 Hz, 1H), 7.28 (m, 6H), 4.18 (q, *J* = 3Hz, 2H), 3.48 (s, 2H), 3.05 (m, 4H), 2.54 (s, 3H), 2.06 (m, 2H), 1.6–1.8 (m, 4H), 1.2–1.5 (m, 3H), 1.42 (t, *J* = 3Hz, 3H). The oil was dissolved in EtOAc, and to this solution was added dropwise a solution of HCl dissolved in ether. The resulting precipitate was filtered and triturated with hexanes to yield 0.105 g of the hydrochloride salt of ketone **6c** as a hygroscopic white solid: mp = 165–167 °C; MS 389.2 (p), 298.0 (p – 91), 172.0 (p – 217), 90.9 (p – 298, base peak). Anal. (C<sub>25</sub>H<sub>31</sub>N<sub>3</sub>O·HCl) C, H, N.

**3-(1-Benzylpiperidin-4-yl)-1-phenylpropan-1-one **6a**:** similarly prepared, free base; MS *m/e* = 308 (M<sup>+</sup>); TLC (1:1 EtOAc:hexanes) *R<sub>f</sub>* = 0.68; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.88 (d, *J* = 3 Hz, 2H), 7.2–7.56 (m, 8H), 3.80 (s, 2H), 2.85–3.20 (m, 4H), 2.20 (brt, *J* = 4 Hz, 2H), 1.40–1.80 (m, 7H).

**3-(1-Benzylpiperidin-4-yl)-1-phenylpropan-1-one hydrochloride, **6a**:** mp = 192–194 °C. Anal. (C<sub>21</sub>H<sub>25</sub>NO·HCl) C, H, N.

**3-(1-Benzylpiperidin-4-yl)-1-(2,3-dihydrobenzo[1,4]-dioxin-6-yl)propan-1-one, **6b**:** free base; mp 110–112 °C; TLC (10:1 CHCl<sub>3</sub>:CH<sub>3</sub>OH) *R<sub>f</sub>* = 0.58; HRMS calcd for C<sub>23</sub>H<sub>27</sub>NO<sub>3</sub> *m/e* = 365.19907 (0.06 ppm); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.44 (s, 1H), 7.43 (d, *J* = 3Hz, 1H), 6.86 (d, *J* = 3Hz, 1H), 4.25 (m, 4H), 3.52 (s, 2H), 2.87 (m, 4H), 1.92 (m, 2H), 1.64 (m, 4H), 1.24 (m, 3H).

**1-(2-Methyl-6-benzothiazolyl)-3-(*N*-benzyl-4-piperidinyl)propan-1-one, **6d**:** free base; mp = 125–127 °C; TLC (10:1 CHCl<sub>3</sub>:CH<sub>3</sub>OH) *R<sub>f</sub>* = 0.61; MS *m/e* = 379.2 (M<sup>+</sup>); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 202.1, 171.0, 156.2, 138.3, 136.0, 133.5, 129.2, 128.2, 127.0, 125.9, 122.3, 122.0, 63.4, 53.7 (2), 36.1, 35.4, 32.2 (2), 30.9, 20.5; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 8.45 (s, 1H), 8.02 (dd, 2H), 7.32 (m, 5H), 3.48 (s, 2H), 3.02 (t, *J* = 7Hz, 2H), 2.86 (m, 2H), 2.85 (s, 3H), 1.93 (m, 2H), 1.68 (m, 4H), 1.31 (m, 3H).

**1-(2-Methyl-6-benzothiazolyl)-3-(*N*-benzyl-4-piperidinyl)propan-1-one hydrochloride, **6d**:** mp = 189–190 °C. Anal. (C<sub>23</sub>H<sub>26</sub>N<sub>2</sub>OS·HCl) C, H, N.

**Preparation of *N*-Acetyl-3-nitro-4-aminoacetophenone, **13**.** To 10 mL of fuming nitric acid cooled to 0 °C was added portionwise 1.0 g (5.6 mmol) of acetophenone derivative **11**. It is important that the temperature be maintained at <5 °C to prevent excess nitration of the benzene ring. The solution was stirred for 15 min at 0 °C and then carefully poured onto ice. A yellow solid precipitated and was collected by filtration to yield 0.42 g (34%) of ketone **13**: mp = 140–141 °C; TLC (2:1 CHCl<sub>3</sub>:EtOAc) *R<sub>f</sub>* = 0.78; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 8.9 (d, 1H), 8.77 (s, 1H), 8.16 (s, 1H), 2.64 (s, 3H), 2.34 (s, 3H).

**Preparation of *N*-Benzoyl-3-nitro-4-aminoacetophenone, **14**.** To 10 mL of fuming nitric acid cooled to –5 °C was added portionwise 2.5 g (10 mmol) of ketone derivative **12**, being certain that the temperature was maintained at <0 °C. The reaction mixture was stirred for 10 min and the resulting solution poured onto ice. A yellow solid precipitate was formed which was collected by filtration. The solid was dissolved in CHCl<sub>3</sub> and chromatographed on silica gel using CHCl<sub>3</sub> as an eluant. Appropriate fractions were combined and evaporated to yield 1.0 g (35%) of ketone **14** as a yellow solid: mp = 235–237 °C; TLC (30:1 CHCl<sub>3</sub>:EtOAc) *R<sub>f</sub>* = 0.8; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 9.12 (d, 1H), 8.84 (s, 1H), 8.25 (d, 1H), 7.96 (d, 2H), 7.6 (m, 3H), 2.66 (s, 3H).

**Preparation of 3-(*N*-Benzyl-4-piperidinyl)-1-(3-nitro-4-acetamidophenyl)-2-propen-1-one, **15**.** A solution of 2.6 g (11.7 mmol) of ketone **13** in 25 mL of THF was cooled to –60 °C under a nitrogen atmosphere. To this was added 4.7 mL (11.7 mmol) of *n*-butyllithium (2.5 M in hexanes) maintaining the temperature <–60 °C. The reaction mixture was stirred for 15 min. A solution of aldehyde **8**, dissolved in 5 mL of THF, was added dropwise maintaining the reaction temperature <–55 °C. The reaction mixture was stirred for 1 h and then warmed to room temperature. At room temperature, the reaction was quenched with 10 mL of water and the mixture extracted with EtOAc. The EtOAc extracts were combined, dried (Na<sub>2</sub>SO<sub>4</sub>), and evaporated to yield a dark oil. This oil was chromatographed on silica gel using 5:1 CHCl<sub>3</sub>:EtOAc as the eluant. Appropriate fractions were combined to yield 1.2 g (25%) of ketone **15** as an oil: TLC (10:1 CHCl<sub>3</sub>:CH<sub>3</sub>OH) *R<sub>f</sub>* = 0.45; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 8.90 (d, 1H), 8.76 (s, 1H), 8.14 (d, 1H), 8.30 (m, 5H), 3.53 (s, 2H), 2.94 (m, 2H), 2.32 (s, 3H), 1.5–2.15 (m, 5H).

**Preparation of 3-(*N*-Benzyl-4-piperidinyl)-1-(3-nitro-4-benzamidophenyl)-2-propen-1-one, **16**.** A solution of 0.80 g (2.9 mmol) of ketone **14** in 30 mL of anhydrous THF was cooled to –70 °C under a nitrogen atmosphere. To this was added 1.2 mL (2.9 mmol) of *n*-butyllithium (2.5 M solution in hexanes) dropwise, forming a dark solution. This solution was stirred at –70 °C for 10 min. To this mixture was added dropwise a solution of 0.6 g (2.9 mmol) of aldehyde **8** in 10 mL of THF. The reaction mixture was slowly warmed to room temperature and stirred for 18 h. The reaction was quenched with 25 mL of water and the mixture extracted with EtOAc. The EtOAc extracts were dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated. The residue was chromatographed on silica gel using 1:1 CHCl<sub>3</sub>:EtOAc as an eluant. Appropriate fractions were combined to yield 0.45 g (34%) of ketone **16**: mp = 150–152 °C; TLC (10:1 CHCl<sub>3</sub>:CH<sub>3</sub>OH) *R<sub>f</sub>* = 0.67; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 9.14 (d, 1H), 8.82 (s, 1H), 8.22 (d, 1H), 7.98 (d, 2H), 7.55 (m, 3H), 7.32 (m, 5H), 7.10 (m, 1H), 6.85 (m, 1H), 3.54 (s, 1H), 2.95 (m, 2H), 1.4–2.3 (m, 7H).

**Preparation of 1-(3-Amino-4-acetamidophenyl)-3-(*N*-benzyl-4-piperidinyl)propan-1-one, **17**.** To a solution of 0.9 g (2.2 mmol) of ketone **15** in 50 mL of ethanol was added 20 mg of PtO<sub>2</sub>, and the mixture was hydrogenated at 50 psi for 1 h. The mixture was filtered and the ethanol evaporated to yield 0.9 g (100%) of ketone **17** as an oil: MS 379.2 (p), 202.3 (p – 176.9), 172.3 (p – 206.9), 91.0 (p – 288.3, base peak); TLC (10:1:0.1 CHCl<sub>3</sub>:CH<sub>3</sub>OH:NH<sub>4</sub>OH) *R<sub>f</sub>* = 0.32; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.6 (s, 1H), 7.2–7.5 (m, 7H), 3.5 (s, 2H), 2.85 (m, 4H), 2.21 (s, 3H), 1.2–2.0 (m, 9H).

**Preparation of 1-(2-Methyl-5-benzimidazolyl)-3-(*N*-benzyl-4-piperidinyl)propan-1-one Hydrochloride, **19**.** A solution of 0.6 g (1.6 mmol) of ketone **17** in 10 mL of acetic acid was heated on a steam bath (80–90 °C) for 1 h. The acetic acid was evaporated and the residue dissolved in 25 mL of EtOAc. To this was added 25 mL of water, and the pH was adjusted to 3.0. The EtOAc layer was separated from the water layer and the water layer then sequentially adjusted to pH = 5.0, 6.0, and 9.0, each time extracting with EtOAc. The pH = 9.0 EtOAc extract was dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated to afford 0.4 g (69%) of ketone **19** (amorphous solid) as the free base: MS *m/e* = 361.3 (p), 270.2 (p – 91.1), 172.3 (p – 189), 91.1 (p – 270.2, base peak); TLC (10:1:0.1 CHCl<sub>3</sub>:CH<sub>3</sub>OH:NH<sub>4</sub>OH) *R<sub>f</sub>* = 0.50; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 8.08 (s, 1H), 7.80 (s, 1H), 7.47 (m, 1H), 7.25 (m, 6H), 3.47 (s, 2H), 2.8–3.0 (m, 4H), 2.59 (s, 3H), 1.90 (t, 2H), 1.64 (m, 4H), 1.25 (m, 3H). The amorphous solid **19** was dissolved in EtOAc, and to this was added an ether solution of HCl. The resulting precipitate was filtered and dried to yield 0.26 g (62%) of the hydrochloride salt of ketone **19** as a tan solid: mp = 123–125 °C dec. Anal. (C<sub>23</sub>H<sub>27</sub>N<sub>3</sub>O·HCl) C, H, N.

**Preparation of 1-(3-Amino-4-benzamidophenyl)-3-(*N*-benzyl-4-piperidinyl)propan-1-one, **18**.** To a solution of 0.45 g (1.0 mmol) of ketone **16** in 50 mL of ethanol was added 25 mg of PtO<sub>2</sub>, and the mixture was hydrogenated at 50 psi for 1 h. After filtration to remove the catalyst, the ethanol was evaporated to yield ketone **18** as an amorphous solid: <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 8.15 (s, 1H), 7.90 (d, 2H), 7.2–7.7 (m, 10H),

3.88 (brs, 2H), 3.50 (s, 2H), 2.90 (m, 4H), 1.2–2.0 (m, 9H). This material was used for the synthesis of **20** without further purification.

**Preparation of 1-(2-Phenyl-5-benzimidazolyl)-3-(N-benzyl-4-piperidinyl)propan-1-one Hydrochloride, **20**.** Ketone **18** was dissolved in a 50:50 mixture of ethanol and acetic acid and heated to 75 °C for 3 h. The reaction mixture was cooled to room temperature and diluted with water. The pH of the mixture was adjusted to 9.5 and the mixture extracted with EtOAc. The EtOAc extracts were dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated to yield 0.19 g (45%) of ketone **20** (free base): MS 424 (M<sup>+</sup>); TLC (10:1:0.1 CHCl<sub>3</sub>:CH<sub>3</sub>OH:NH<sub>4</sub>OH) R<sub>f</sub> = 0.40; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 8.14 (d, 2H), 7.86 (d, 1H), 7.2–7.6 (m, 11H), 3.58 (s, 2H), 2.92 (m, 4H), 1.2–2.1 (m, 9H). The residue was dissolved in EtOAc, and to this solution was added dropwise an ether solution of HCl. The resulting precipitate was collected via filtration and dried to yield the hydrochloride salt of **20** as a tan solid: mp > 300 °C. Anal. (C<sub>28</sub>H<sub>29</sub>N<sub>3</sub>O·HCl) C, H, N.

**Inhibition of Acetylcholinesterase and Butyrylcholinesterase.** The method of Ellman *et al.*<sup>18</sup> was followed. The assay solution consisted of a 0.1 M sodium phosphate buffer, pH = 8.0, with the addition of 100 μM tetraisopropylpyrophosphoramide (*iso*-OmpA), 100 μM 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), 0.02 unit/mL AChE (Sigma Chemical Co.; from human erythrocytes), and 200 μM acetylthiocholine iodide. The final assay volume was 0.25 mL. Test compounds were added to the assay solution prior to enzyme addition, whereupon a 20-min preincubation period with enzyme was followed by addition of substrate. Changes in absorbance at 412 nM were recorded for 5 min. The reaction rates were compared, and the percent inhibition due to the presence of test compounds was calculated.

Inhibition of butyrylcholinesterase was measured as described above for AChE by omitting addition of *iso*-OmpA and substituting 0.02 unit/mL BuChE (Sigma Chemical Co.; from human serum) and 200 μM butyrylthiocholine for enzyme and substrate, respectively.

**Measurement of Elevation of ACh in Mouse Brain.** AChE inhibitors were given orally. Animals were sacrificed by focused microwave irradiation at 1 h postdosing, and the forebrains were removed and homogenized in 20 mM sodium phosphate buffer, pH = 5.3. Homogenates were centrifuged 20 min at 12000g; supernatants (10–20 μL) were used for determination of ACh with the ACh analysis system from Bioanalytical Systems (West Lafayette, IN). A polymeric anion-exchanged column resolves ACh from choline (Ch), and a postcolumn reactor column containing immobilized AChE and choline oxidase converts ACh and Ch to betaine and hydrogen peroxide; the hydrogen peroxide is readily measured with an electrochemical detector at 500 mV vs Ag/AgCl reference electrode and a platinum working electrode. Sensitivity was approximately 3–5 pmol of ACh. Typical ACh control values were 18–25 nmol/g in mouse forebrain. Data for drug treated animals are reported as percent control values. In general, the treatment consisted of eight animals per group. Statistical significance was determined by Student's one-tailed *t*-test.

**In Vivo Microdialysis.** Male Sprague–Dawley rats were implanted in the corpus striatum with guide cannulae and dialysis probes (Bioanalytical Systems, West Lafayette, IN) and superfused at a rate of 3 μL/min. The dialysis fluid was a Ringer's buffer (pH = 7.2) containing 500 nM physostigmine to reduce degradation of ACh by AChE. Fractions (60 μL) were collected every 20 min for 2 h before drug administration and for 3 h following oral administration of drug. Samples (50 μL) were used directly for HPLC analysis of ACh content as described above. Basal ACh release was defined as the average ACh content in the three fractions just prior to drug administration. ACh content in all fractions was converted to a percentage of these basal values; thus, each animal served as its own control.

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## References

- (1) Perry, E. K. The Cholinergic hypothesis - Ten Years On. *Br. Med. Bull.* **1986**, *42*, 63–69 and references cited therein.
- (2) Bartus, R. T.; Dean, R. L.; Beer, B.; Lippa, A. S. The Cholinergic Hypothesis of Geriatric Memory Dysfunction. *Science* **1982**, *217*, 408–417.
- (3) Davis, K. L.; Mohs, R. C.; Tinklenberg, J. R. Enhancement of Memory by Physostigmine. *N. Engl. J. Med.* **1979**, *301*, 946.
- (4) (a) Summers, W. K.; Majovsky, L. V.; Marsh, G. M.; Tachiki, K.; King, A. Oral Tetrahydroaminoacridine in Long-Term Treatment of Senile dementia, Alzheimers Type. *N. Engl. J. Med.* **1986**, *315*, 1241–1245. (b) Eagger, S. A.; Levy, R.; Sahakian, B. J. Tacrine in Alzheimers Disease. *Lancet* **1991**, *337*, 889–892. (c) Watkins, P. B.; Zimmerman, H. J.; Knapp, M. J.; Gracon, S. I.; Lewis, K. W. Hepatotoxic Effects of Tacrine Administration in Patients With Alzheimer's Disease. *J. Am. Med. Assoc.* **1994**, *271*, 992–998.
- (5) (a) E-2020. *Drugs Future* **1991**, *16*, 16–18. (b) Sugimoto, H.; Imura, Y.; Yamanishi, Y.; Yamatsu, K. Synthesis and Anti-acetylcholinesterase Activity of 1-Benzyl-4-[(5,6-dimethoxy-1-indanon-2-yl)methyl]piperidine hydrochloride (E2020) and Related Compounds. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 871–876.
- (6) Villalobos, A.; Blake, J. F.; Biggers, C. K.; Butler, T. W.; Chapin, D. S.; Chen, Y. L.; Ives, J. L.; Jones, S. B.; Liston, D. R.; Nagel, A. A.; Nason, D. M.; Nielsen, J. A.; Shalaby, I. A.; White, W. F. Novel Benzisoxazole Derivatives as Potent and Selective Inhibitors of Acetylcholinesterase. *J. Med. Chem.* **1994**, *37*, 2721–2734.
- (7) Atack, J. R.; Yu, Q.-S.; Soncrant, T. T.; Brossi, A.; Rapaport, S. I. Comparative Inhibitory Effects on Various Physostigmine Analogs Against Acetyl- and Butyrylcholinesterases. *J. Pharmacol. Exp. Ther.* **1989**, *249*, 194–202.
- (8) Gibson, M.; Moore, T.; Smith, C. M.; Whelpton, R. Physostigmine Concentrations After Oral Doses. *Lancet* **1985**, 695–696.
- (9) Knapp, S.; Wardlow, M. L.; Albert, K.; Waters, D.; Thal, L. J. Correlation Between Plasma Physostigmine Concentrations and Percentage Acetylcholinesterase Inhibition Over Time After Controlled Release Physostigmine in Volunteer Subjects. *Drug Metab. Dispos.* **1991**, *19*, 400–404.
- (10) Brufani, M.; Marta, M.; Pomponi, M. Acetylcholinesterase Activity of a New Carbamate, Heptylphysostigmine, In View of its Use in Patients with Alzheimer-Type Dementia. *Eur. J. Biochem.* **1986**, *157*, 115–120.
- (11) Thomsen, T.; Zende, B.; Fischer, J. P.; Kewitz, H. *In vitro* effects of various cholinesterase inhibitors on acetyl- and butyrylcholinesterase of healthy volunteers. *Biochem. Pharmacol.* **1991**, *41*, 139–141 and references cited therein.
- (12) Forsyth, D. R.; Wilcock, G. K.; Morgan, R. A.; Truman, C. A.; Ford, J. M.; Roberts, C. J. C. Pharmacokinetics of Tacrine Hydrochloride in Alzheimers Disease. *Clin. Pharmacol. Ther.* **1989**, *46*, 634–641. Truman, C. A.; Ford, J. M.; Roberts, C. J. C. Comparison of the Chromatographic Characteristics of Tacrine Hydrochloride in Human Serum and Urine with Those of *In Vitro* Metabolic Products from Hepatic Microsomes. *Biochem. Pharmacol.* **1991**, *42*, 956–959.
- (13) Mihara, M.; Ohnishi, A.; Tomono, Y.; Hasegawa, J.; Shimamura, Y.; Yamazaki, K.; Morishita, N. Pharmacokinetics of E2020, a New Compound for Alzheimer's Disease, in Healthy Male Volunteers. *Int. J. Clin. Pharmacol. Toxicol.* **1993**, *31*, 223–229.
- (14) Haginaka, J.; Seyama, C. Determination of Enantiomers of 1-Benzyl-4-[5,6-dimethoxy-1-indanon-2-yl]methylpiperidine Hydrochloride (E2020), a Centrally Acting ACh Esterase Inhibitor, in Plasma by Liquid Chromatography with Fluorometric Detection. *J. Chromatogr.* **1992**, *577*, 95–102.
- (15) Hermans, B.; VanDeele, P. Synthesis of 1-Benzyl-4-formylpiperidine. *Ind. Chim. Belge* **1967**, *32*, 64–65.
- (16) Burgess, E. M.; Penton, H. R.; Taylor, E. A. Thermal Reactions of Alkyl N-Carbomethoxyulfamate Esters. *J. Org. Chem.* **1973**, *38*, 26–31.
- (17) Sawhney, S. N.; Singh, J.; Bansal, O. P. Synthesis and Anti-inflammatory Activity of some 6-[2-Amino-(and substituted amino)-4-thiazolyl] benzothiazoles. *J. Ind. Chem. Soc.* **1975**, *52*, 561–562.
- (18) Ellman, G.; Courtney, K. D.; Andrews, V.; Featherstone, R. M. A New and Rapid Colorimetric Determination of Acetylcholinesterase Activity. *Biochem. Pharmacol.* **1961**, *7*, 88.